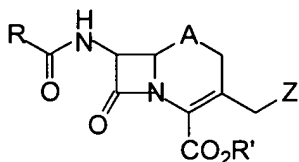


IN THE CLAIMS

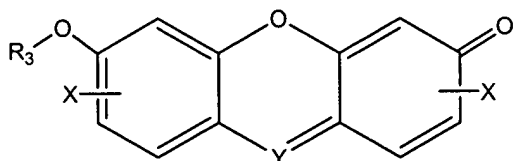
1. (Currently amended) A compound having the ~~general~~ formula:



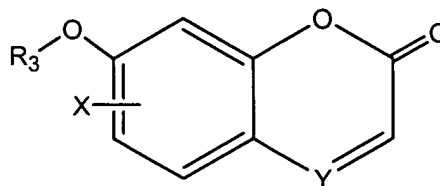
(I)

in which R is a benzyl, 2-thienylmethyl, or cyanomethyl group; R' is selected from the group consisting of H, alkyl, physiologically acceptable salts or metal, ~~ester groups~~, ammonium cations, $--CHR_2OCO(CH_2)_nCH_3$, $--CHR_2OCOC(CH_3)_3$, in which R₂ is selected from the group consisting of H and lower alkyl, acylthiomethyl, acyloxy-alpha-benzyl, deltabutyrolactonyl, methoxycarbonyloxymethyl, phenyl, methylsulphinylmethyl, β -morpholinoethyl, dialkylaminoethyl, and dialkylaminocarbonyloxymethyl, and n is from 1-4; A is selected from the group consisting of S, O, SO, SO₂ and CH₂; and Z is a donor fluorescent moiety.

2. (Currently amended) The compound of claim 1, wherein the donor fluorescent moiety is selected from the group consisting of:



(II)



(III)



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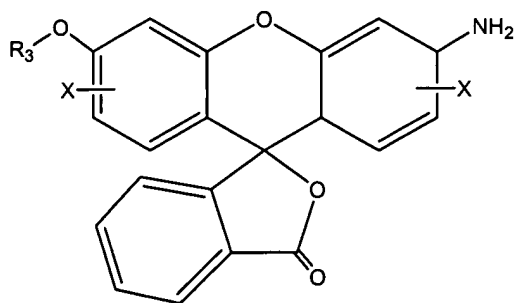
Applicant: Tsien and Rao

Filed: January 11, 2002

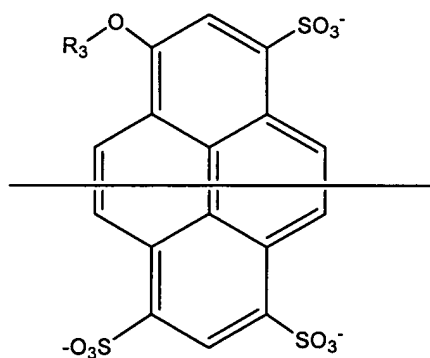
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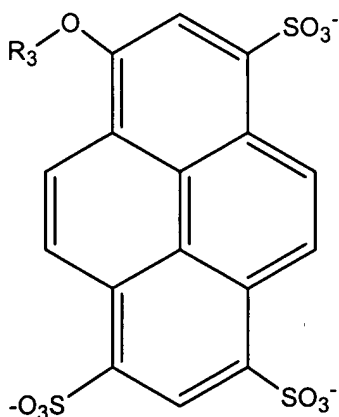
Attorney Docket No.: REGEN1510-1



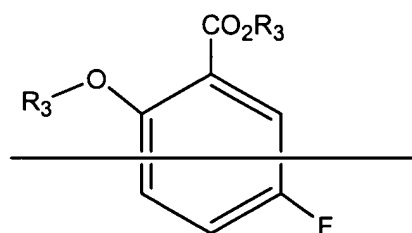
(IV)



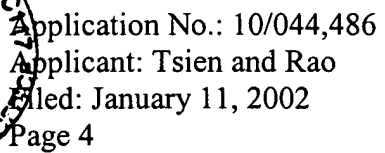
(V)



(V)



(VI)



PATENT





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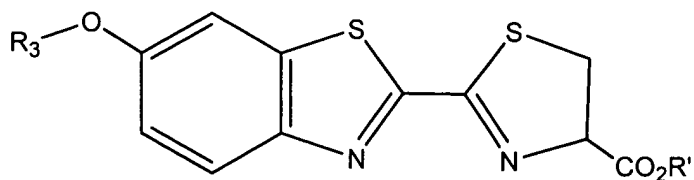
Applicant: Tsien and Rao

Filed: January 11, 2002

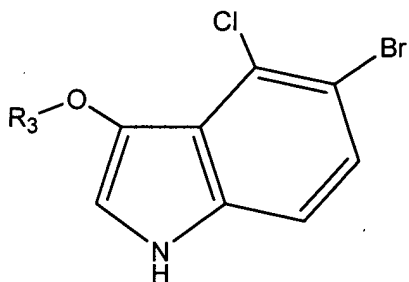
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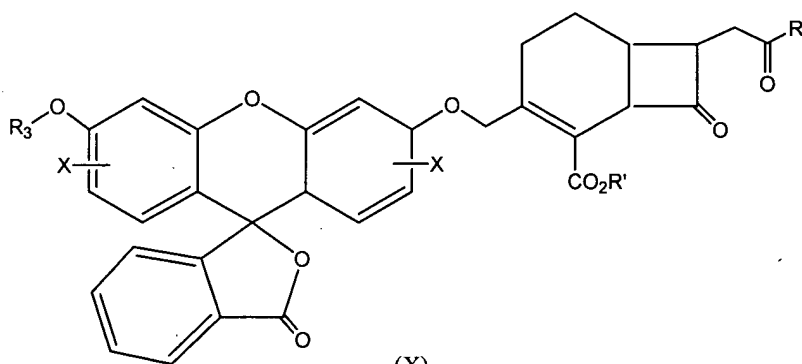
Attorney Docket No.: REGEN1510-1



(VIII)



(IX)



(X)



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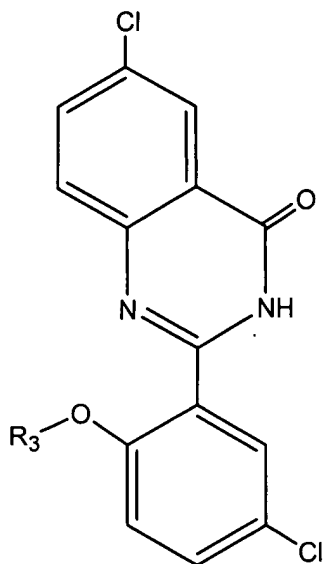
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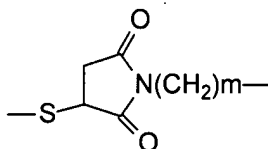
Attorney Docket No.: REGEN1510-1



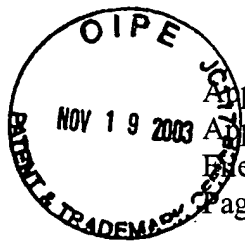
(XI)

wherein R and R' are as defined in claim 1, R₃ is a linker for the fluorescent donor, X is H, F, Cl, Br, or CO₂R', ~~or lower alkyl~~, and Y is N, CH, C-CN, or C-CF₃ ~~or O~~.

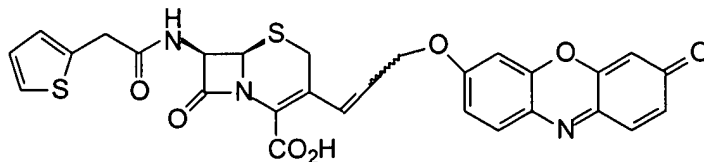
3. (Currently amended) The compound of claim 2, wherein the linker is selected from the group consisting of a direct bond to a heteroatom in the fluorescent moiety, --O(CH₂)_n--, --S(CH₂)_n--, --NR₂(CH₂)_n--, --N⁺R₂(CH₂)_n--, --OCONR₂(CH₂)_n--, --O₂C(CH₂)_n--, --SCSNR₂(CH₂)_n--, --SCSO(CH₂)_n--, --S(CH₂)_nCONR₂(CH₂)_m--, --S(CH₂)_nNR₂CO(CH₂)_m, and



in which R₂ is as previously defined; and m and n are each independently integers from [0] 1 to 4.

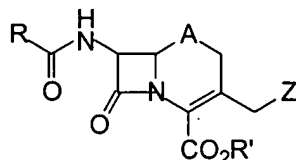


4. (Currently amended) A ~~The compound of claim 1, wherein the compound has~~
having the structure:



5. (Withdrawn) A method for detecting the presence of β -lactamase activity in a sample, comprising:

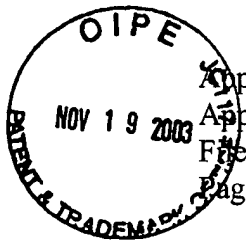
contacting the sample with at least one compound of general formula I:



(I)

in which R is a benzyl, 2-thienylmethyl, or cyanomethyl group, or a quencher; R' is selected from the group consisting of H, physiologically acceptable salts or metal, ester groups, ammonium cations, --CHR₂OCO(CH₂)_nCH₃, --CHR₂OCOC(CH₃)₃, in which R₂ is selected from the group consisting of H and lower alkyl, acylthiomethyl, acyloxy-alpha-benzyl, deltabutyrolactonyl, methoxycarbonyloxymethyl, phenyl, methylsulphinylmethyl, β -morpholinoethyl, dialkylaminoethyl, and dialkylaminocarbonyloxymethyl; A is selected from the group consisting of S, O, SO, SO₂ and CH₂; and Z is a donor fluorescent moiety.

6. (Withdrawn) The method of claim 5, wherein said sample has a β -lactamase reporter gene.



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7. (Withdrawn) The method of claim 6, wherein said β -lactamase reporter gene is in a mammalian cell.
8. (Withdrawn) The method of claim 5, wherein samples having β -lactamase activity are separated from samples having no β -lactamase activity by fluorescent-activated cell sorting.
9. (Withdrawn) The method of claim 5, wherein the β -lactamase activity results from a β -lactamase enzyme that was prepared by mutagenesis of another β -lactamase enzyme.
10. (Withdrawn) The method of claim 5, wherein said compound is a membrane permeant derivative.
11. (Withdrawn) The method of claim 5, wherein the donor fluorescent moiety is selected from the group consisting of:



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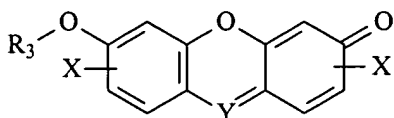
Applicant: Tsien and Rao

Filed: January 11, 2002

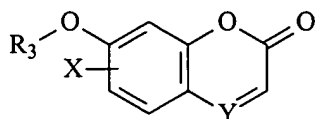
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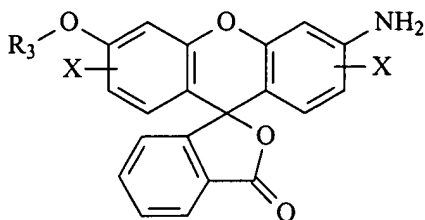
Attorney Docket No.: REGEN1510-1



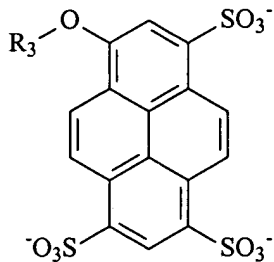
(II)



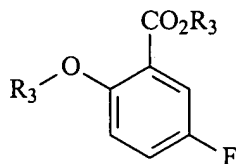
(III)



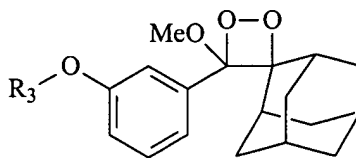
(IV)



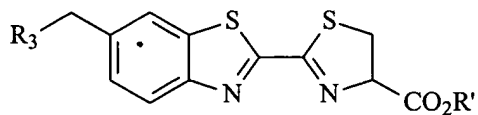
(V)



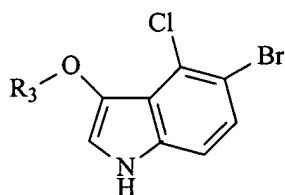
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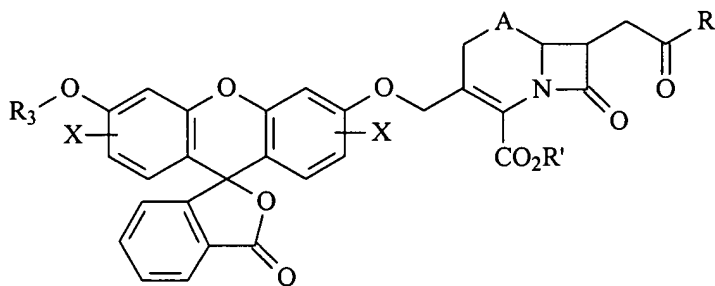
(VII)



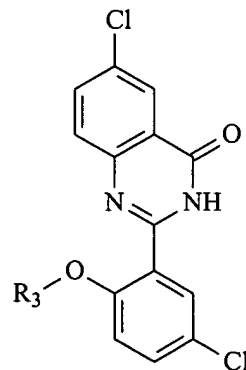
(VIII)



(IX)



(X)



(XI)

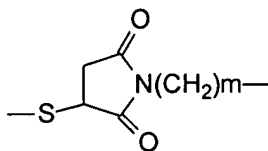


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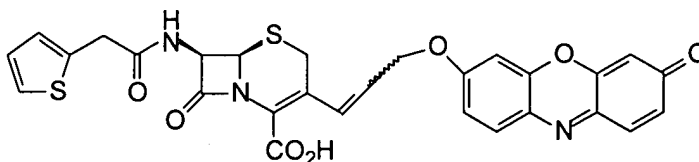
R_3 is a linker for the fluorescent donor, X is H or lower alkyl, and Y is N or O.

12. (Withdrawn) The method of claim 11, wherein the linker is selected from the group consisting of a direct bond to a heteroatom in the fluorescent moiety, $--O(CH_2)_n--$, $--S(CH_2)_n--$, $--NR_2(CH_2)_n--$, $--N^+R_2(CH_2)_n--$, $--OCONR_2(CH_2)_n--$, $--O_2C(CH_2)_n--$, $--SCSNR_2(CH_2)_n--$, $--SCSO(CH_2)_n--$, $--S(CH_2)_nCONR_2(CH_2)_m$, $--S(CH_2)_nNR_2CO(CH_2)_m$, and



in which R_2 is as previously defined; and m and n are each independently integers from 0 to 4.

13. (Withdrawn) The method of claim 5, wherein the compound has the structure:



14. (Withdrawn) A method for determining whether a compound of claim 1 is a substrate for a β -lactamase enzyme, comprising: contacting said compound with a sample containing said β -lactamase enzyme; exciting at the wavelength for the said compound when cleaved; and measuring fluorescence.

15. (Withdrawn) The method of claim 14, wherein said compound is a membrane permeant derivative.

16. (Withdrawn) The method of claim 14, wherein said β -lactamase enzyme has been prepared by mutagenesis of another β -lactamase enzyme.